Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

APOGAMY IN ATAMOSCO

LULA PACE

(WITH PLATES XIII AND XIV)

Atamosco texana Greene (*Zephyranthes texana*) is one of the Amaryllidaceae, and is called amaryllis, atamosco lily, or rain lily. The so-called rain lilies in this region bloom in the summer and autumn for a few days after each rain. This one is not quite so well known here as the white lily, *Cooperia Drummondii*, which is about twice the size and much more abundant in this locality; but 100–200 miles farther west *Atamosco* is the more plentiful.

The first collection of material was made for class use, as the ovules and embryo sac are very large; but very early in the preparation of this material interesting things were noticed. So the material on hand was carried to Bonn and worked over there so far as the stages permitted. I regret exceedingly not being able to complete it at that time under the direction and with the suggestions and criticisms of Professor STRASBURGER. But it gives me great pleasure to acknowledge my indebtedness to him for his kindly interest and stimulating suggestions during the first part of the work.

Material

The material was all collected in one locality, near Waco, Texas; and in this particular place *Atamosco* is quite abundant over two or three acres. But as the flowers are solitary and often precede the leaves, it is not easy to find young stages. I now have plants under cultivation and hope to get these desired stages later.

Two collections have been made, one in 1909 and the other in 1911. The intervening year I was abroad and no collection could be made. The material was fixed in chromacetic acid (0.25 per cent chromic acid and 1 per cent acetic acid). In some cases about 10 drops of 1 per cent osmic acid was added to 50 cc. of the above. Alcohol-acetic (three parts absolute alcohol and one part acetic acid) and alcohol-formalin (two parts of 50 per cent alcohol and one part of 10 per cent formalin) were also used. The triple stain, safranin, gentian violet, and orange G, and iron hematoxylon were found most satisfactory.

Pollen

The pollen is quite normal in every way in the anthers sectioned (fig. 1). The tube nucleus is usually somewhat amoeboid in shape and occasionally has more than one nucleolus. The generative cell with its nucleus and bit of cytoplasm about it is quite small. In this figure the generative nucleus has the chromosomes segmented. The number is 12 and was counted in several other grains in the same anther. But in the majority of the pollen grains studied the generative nucleus was in the spirem stage (fig. 2). In this same anther a few grains were seen with possibly two generative nuclei; but it is probable they are the sperm nuclei, as they are both in the same cell. A germinating pollen grain from the stigma shows the generative nucleus in mitosis and in advance of the tube nucleus (fig. 3). In fig. 3, *a* this nucleus is shown with greater magnification, and the remainder of this nucleus, which was in the adjacent section, is shown in *b*. The chromosomes show longitudinal splitting and there are 12.

Embryo sac

The mother cell is only slightly larger than the adjacent cells when first distinguishable (fig. 4). The ovule now increases rapidly in size and the mother cell becomes very large. The division of the mother cell has not been seen. Many ovaries with ovules showing large mother cells have been cut, and many that were apparently the same size had mature sacs. The development of the sac must be very rapid, for several ovaries had mature sacs and mother cells apparently occurring together without any definite order. But other ovaries showed all ovules in the same stage of development; one in particular has every ovule with the nuclei of its sac in the third and last division, with all stages of mitosis from well developed spirem to late telophase.

The two-nucleate (fig. 5) and four-nucleate sacs show nothing unusual. The spirem for the next division has the normal appearance. In figs. 6 and 6, *a* the anaphase stage of the two nuclei from the micropylar end of a four-nucleate sac are shown. The entire figures being found in two adjacent sections, both are drawn separately, as the number of chromosomes is so large that it is difficult to reconstruct the two figures and make one accurate drawing. In one nucleus the spindle is almost parallel with the section. In this spindle at one end 11 chromosomes appear, and the same end in the next section shows 13, giving 24 chromosomes at one end of this spindle. It is not possible to make an accurate count at the other end, but there seems to be about the same number. The other nucleus is almost perpendicular to the section and at one end the 24 chromosomes are unusually distinct. A metaphase from the chalazal end of another four-nucleate sac is shown in fig. 7. It also shows the 24 chromosomes. A similar one might be given from the micropylar end of another sac. A somatic cell taken from the walls of the ovule is given for comparison (fig. 8); it shows 24 chromosomes. Fig. 9 is a telophase of this same division from the micropylar end of the sac. The old spindle of the previous division is still quite distinct, but less so than the spindle of this division. This spindle shows wall beginnings in the one parallel with the sections. Only one end of the other spindle appears in this section, as it is perpendicular to it. But this nucleus, which already has the nuclear membrane developed about it, shows approximately 24 chromosomes.

The 8 nuclei arrange themselves in the usual fashion, egg apparatus, antipodals, and two polars that fuse, each group being very distinct and almost diagrammatically organized. The filiform apparatus is well developed, but not so strikingly as in *Parnassia* (**20 a**). The indentations so noticeable in the *Parnassia* synergids are not often seen at all in *Atamosco* (fig. 10). It was present in a few instances, but was never a deep notch. The only noticeable feature of the sac is the large nuclei containing little other stainable material than the large nucleolus. There are often two nucleoli in the egg and occasionally in the synergid nuclei also. They both stain red in the triple stain, or if stained in the iron hematoxylon, they are apparently alike. The antipodal end of the sac has the usual organization (fig. 11). The three antipodal cells are definitely organized, but are without cell walls. The polars are in contact in the center of the sac. Fig. 12 shows the egg apparatus after the pollen tube enters. One male nucleus is within the egg near the nucleus, the other is just below it. The primary endosperm nucleus lies just above the antipodals and the fusion of the polars seems to be complete. Fig. 13 shows about the same stage. In this case one male nucleus is still not in contact with the egg, while the other has already fused with the polars, showing only as a bit of very dense nuclear material in the upper part of the primary endosperm nucleus, in which all the nuclei are still evident (fig. 13, *a*). Somewhat earlier stages were found with the polars still quite distinct and the male nucleus some distance away. The adjacent ovule shows a similar condition to that in fig. 13, *a*. Another sac in which the pollen tube has entered is shown in fig. 14. The sperm nucleus is not in contact with the egg nucleus, but the other sperm nucleus has begun fusing with the almost completely fused polars (fig. 14, *a*). Here both sperm nuclei have very dense masses of chromatin, looking like a very thick spirem except for thin places at intervals. These thin places seem to be only very delicate threads which with slight magnification are not easily seen, and the nuclei then have the appearance of having the chromosomes already segmented. In fig. 15 the micropylar end of a sac is given with the male nucleus just in contact with the egg nucleus, but there is no evidence that it is fusing, not even a flattening of either nucleus. The triple fusion is already completed and the spirem is forming for the first division. One part of this nucleus is very much denser than the remainder of it and is probably where the male nucleus entered. The very large nucleolus seems to be budding. In another sac the male nucleus lies directly in front of the egg nucleus, but is not in contact with it, while the primary endosperm nucleus of this sac has completed the first division. Here the wall has formed, but it quickly disappears, for the endosperm contains many nuclei before permanent walls appear. The male nucleus does not always come in contact with the egg nucleus, or if it does, it moves away, as it is not in contact in older stages (figs. 17–23).

About 600 sacs have been seen in which the sperm nucleus is within the egg. In very few instances are they in contact, and these were always the earlier stages. The flowers probably always wither within 24 hours after opening. Those brought in and pollinated had all done so. These withered flowers show the male nucleus within the egg.

Fig. 16 shows an early prophase in the division of the egg. The spirem develops in the usual way, and is very long. Fig. 17 shows the segmentation completed, with a very pale nucleolus and approximately 24 chromosomes. The male nucleus is at some distance from the egg nucleus and shows no change. Fig. 19 shows a similar stage. In Fig. 20 the nuclear membrane has disappeared. About this stage the male nucleus begins to disintegrate (fig. 18); but sometimes the spindle is forming with the membrane of the male nucleus apparently still intact (fig. 21). A spindle is shown in fig. 22, and here the male nucleus is disorganizing. A late anaphase of the egg has the male nucleus entirely without a membrane (fig. 23). The chromatic material is organized into approximately 12 masses, which seem to be more or less perfectly formed chromosomes, although there are evident signs of disintegration. There is nothing in the egg or in this nucleus itself to suggest the possibility of its being a cell of the embryo. This suggests the possibility of "merogonie."

A somewhat earlier anaphase shows approximately 24 chromosomes at each end of the spindle, and the somewhat elongated male nucleus apparently in contact with the spindle (fig. 24). This sac has four endosperm nuclei, two near the base of the sac and two near the middle. Fig. 25 is that of an undivided egg. The pollen tube has the three nuclei in it. There are about 64 endosperm nuclei in the sac and but little trace of the synergids in the adjacent sections. Other sacs in this ovary have embryos with about 16 cells. It is probably a case of a delayed pollen tube and the primary endosperm nucleus dividing before fusion with the male nucleus, although this is the only instance seen where that seems probable. But as has been said above, most of the material of this stage was pollinated in order to be sure of fertilization. It is also possible for this to be the second pollen tube to enter this sac. But if so, all trace of the other one has disappeared.

The endosperm nuclei often contain 7 or 8 nucleoli, all of which stain a brilliant red in the safranin–gentian-violet combination.

An endosperm tissue 2–4 cells in width, but which does not completely fill the sac, and with many free nuclei still present, is found with the embryo of 30–40 cells. There is such a mass of long curved chromosomes in the endosperm that no satisfactory count could be made from any of my preparations. In several instances one could count 50 and know there were still others. One such nucleus, which was in three adjacent sections, is shown in figs. 26, 26, *a*, and 26, *b*. It seems clearer to show each section separately than to reconstruct the nucleus from the three sections. Here more than 60 chromosomes can be counted in all three sections, but it is certain that there were several cut chromosomes, as some were evidently shorter than others, and in this plant the chromosomes all seem to be approximately the same length. The number would be 60 from the fusion of two polars with 24 each and a sperm nucleus with 12. It is possible that in some cases the polars only enter into the fusion, which would give 48. But the triple fusion seems the common condition in the material examined. This is probably the fourth division of the endosperm of this sac. These divisions are not always exactly simultaneous; for in one instance in the second division one nucleus has completed the mitosis, the new nuclei being already perfect, while the other nucleus was still in the anaphase.

Embryo

The embryo shows nothing unusual in its development. The first division has already been described, and is shown in figs. 20–24. One two-celled embryo was seen with a vertical wall, but in all others it was the usual horizontal wall (fig. 27). This figure shows the pollen tube entering one synergid.

Abnormal sacs

Atamosco also has many sacs in which the arrangement of the nuclei does not follow the usual form. These sacs have the antipodal group in the micropylar end of the sac and egg apparatus at the side. The organization of the two groups of cells is as perfect and as characteristic as if they were in the usual position. About 2000 ovaries have been cut; many were too young to show mature sacs, and of others no record was kept. But in about 300 ovaries that were mature, there were side eggs in 56 and in these 56 ovaries there were 205 of these abnormal cases. The other sacs in these ovaries had nothing unusual in their nuclear arrangement. In the majority of these abnormal sacs there was nothing unusual except the position of the cells, and only those with cells in unusual positions were included in the above statements.

Fig. 28, however, shows two eggs in the micropylar end of the sac with no synergids. The whole of this sac was not seen, so just what the arrangement of the other cells was could not be determined. Both of these eggs may develop, for a sac was seen with two young embryos, one of about 6 and the other of 10 cells, located exactly as these two egg cells were, and with endosperm of 30–40 nuclei. Two other sacs with embryos similar to this were seen. Occasionally more than two eggs may be organized in a sac (fig. 29). This sac has 5 cells in the micropylar end, all of which look like eggs, and two of which show intermediate characters and may function as synergids. This leaves only one antipodal and two polars which have already fused and lie very near the antipodal. Two eggs and two synergids are sometimes found in the one egg apparatus. Sometimes more than one egg apparatus is organized. One was seen with two at the side of the sac, in addition to the usual micropylar apparatus. Not all of this sac was seen, but it has at least 12 nuclei in it. The egg apparatus in these side positions is usually normal in all other particulars; but in one case in which it is at the side near the antipodal end of the sac, the egg is turned vertically, while the synergids have themselves oriented so as to bring their apices toward the edge of the sac.

What might be called a double sac is shown in fig. 30. There are 15 nuclei present, and it is possible there should be 7 instead of 6 in the micropylar group, making 16 altogether, but in tracing from section to section I could not be quite sure. Fig. 31 is from a sac that is almost normal. It seems to have 4 antipodals, and an egg apparatus of one synergid and one egg some little distance from the micropylar end of the sac. The sperm nucleus is in contact and just behind the egg nucleus. A sperm has probably fused with the polars, as there is a dense portion in this otherwise almost clear nucleus. In many of these sacs the antipodal end contains no nuclei unless the egg apparatus approaches that region. Fig. 32 is from a sac with 13 nuclei, 7 of which have the organization of the antipodals at the micropylar end of the sac, 4 are in the lower part of the sac near the antipodal region, two synergids and two eggs, while the two polars are the only normal feature of the sac. The pollen tube is entering the egg apparatus.

As was said above, in the large majority of these abnormal sacs there are the usual 8 nuclei, and these have the usual organization, but the groups are not in the usual position. Fig. 33 shows a common arrangement except the antipodals here are not exactly at the micropylar end. The egg apparatus is perfect (fig. 33, *a*), as are the polars and antipodals, each group showing its characteristic differentiation. A four-celled embryo in a similar sac is shown in fig. 34. Here the primary endosperm has not divided. PORSCH (21) and those holding the archegonium theory would regard these extra groups as the organization of additional archegonia. If the two groups of cells usually found in an embryo sac are archegonia, the interchange of characteristic organization would not be very remarkable. In the 16-nucleate sac of *Euphorbia procera*, MODILEWSKI (15) figures 4 groups of 4 nuclei each. From each of these groups one nucleus moves to the center to form the primary endosperm nucleus, leaving 3 in each of the groups. The micropylar group he calls the egg apparatus, and that one is the only one figured as functioning. But each of the groups at the side of the sac have the appearance of an egg apparatus.

Discussion

The egg apparatus is almost diagrammatic, so the first thing to attract attention was the very small sperm nucleus as compared with the egg nucleus. But as the egg nucleus contains very little stainable material except the nucleolus, while the sperm nucleus is very dense, the real condition was not suspected, for the sperm nucleus is often much smaller than the egg nucleus. LAND (14) figures a very small but dense spiral sperm in *Silphium*. NAWASCHIN (17) shows a similar one in *Helianthus*. GUIGNARD gives a like condition in *Iris* (7), and in some of the Ranunculaceae (9) and Solanaceae

(10); and STRASBURGER (27) figures a small more or less coiled sperm for *Urtica dioica*. Less difference in the relative size of the two nuclei appears in *Cypripedium* (19). Other instances might be cited, but these are sufficient to show that a difference in the size of the sex nuclei is not unusual.

Older stages were then cut and a spirem was found in the egg nucleus while the sperm was often not even in contact with it. This was supposed to be a case of the early prophases taking place before the fusion of the sex nuclei. GUIGNARD (8) shows such a case in *Lilium Martagon*, and *Cypripedium* (19) and *Calopogon* (20) both show this condition. BLACKMAN (1), CHAMBERLAIN (3), and FERGUSON (6) have all figured early stages in mitosis before the fusion of the sex nuclei in *Pinus*. In all of these, however, the sperm nucleus was as active as that of the egg, both nuclei being in approximately the same stage. But in *Atamosco* only the egg nucleus formed a spirem (fig. 16). It soon began to seem strange that there was no increase in the size of the sperm and no evidence of fusion. So more and more material of this stage was cut. In all cases where the pollen tube had entered the sac this condition was seen. More than 600 examples like figs. 12–15 were found, and about half as many in which the spirem in all stages is formed in the egg nucleus (fig. 16). Two-celled embryos show no trace of this sperm nucleus (fig. 27); and a few spindles and telophase stages of this division also give no trace of it.

Then young ovaries were cut to get the development of the sac. But not many had been collected and no satisfactory chromosome count could be made. In one ovary the third mitosis was in progress, but unfortunately the material was not well fixed, some plasmolysis having taken place. Yet in two nuclei of the metaphase stage more than 12 chromosomes were certainly present; but in both instances the nuclei were in the chalazal end of the sac; so this might only mean a repetition of the condition STRASBURGER (25) found in *Lilium Martagon*, of which he says:

> Die Schwesterchromosomen bei der homöotypischen Teilung des im Chalazaende der Embryosakanlage (die hier direkt aus der Embryosakmutterzelle hervorgeht) befindlichen Kerns, statt wie sonst, ohne Längsspaltung in die Kernspindel eingefügt zu werden, eine solche Längsspaltung, wie bei

> einem typischen Teilungeschritt erfahren, was eine Verdoppelung der Chromosomenzahl zur Folge bat.

This was the state of the problem when I left Bonn.

A new collection was made this last autumn. Even from this collection the division of the mother cell has not been obtained. As was stated above, the development of the sac must go on very rapidly, for in some ovaries certain ovules are in the mother cell stage while others have mature sacs; and these seem not to be related to any part of the ovary or to follow in any special succession; they occur apparently without any order. The buds appear at the surface of the soil one day with the mother cell undivided and the flowers are open the next and usually last only one day; so the whole development must go on very rapidly.

There is no evidence that permanent walls came in at the division of the mother cell. The two-nucleate sac (fig. 5) never shows any evidence of disintegrating megaspores; so it belongs to the *Lilium* type described by COULTER and CHAMBERLAIN (**5**, p. 73), in which these two nuclei are the daughter nuclei without separating walls, and the nuclei of the four-nucleate sac are the four megaspore nuclei without separating walls. A discussion of this question was given in the paper on *Cypripedium* (**19**), and also by COULTER (**4**). A recent paper by BROWN and SHARP (**2**) shows this condition, and the same view is presented.

The third division in the sac I was fortunate enough to get in nearly all the ovules of one ovary. In many of these the chromosomes were quite distinct and several counts were made (figs. 6, 7, 9). In all cases approximately 24 chromosomes could be counted. The sporophyte number is 24 (fig. 8); therefore the reduction division seems not to have taken place. This gives the egg the sporophyte or diploid number of chromosomes; and, according to STRASBURGER'S definition, when such an egg develops an embryo without fertilization it is a case of apogamy. He says (**26**, p. 80):

> Daher ich das Kriterium für echte Parthenogenesis in der Weiterentwicklung eines Geschlectsproduktes erblickte, das mit der ihm normal Zukommenden haploiden Chromosomenzahl anhebt. Wenn ein diploides Ei einen keim bildet, so bezeichnete ich dessen Entwicklung als Apogam, das Ei selbst als apogames Ei.

He also says that such a diploid egg is in reality already fertilized,

> und zwar in dem Sinne, dass der Zustand, den die Befruchtung in seiner Mutterpflanze schuf, in ihm noch fortdauert, da er nicht durch den Vorgang der Reduktionsteilung, gewissermassen durch Entfruchtung, aufgehoben wurde.

It is already from its organization endowed with the double number of chromosomes, so it goes on, so far as the chromosome number is concerned, developing like the cells of a bud or an adventive embryo. The development of these *Anlagen* would hardly be compared to genuine parthenogenesis, in spite of the fact that no fertilization takes place here. But WINKLER used a different terminology, based on a somewhat different conception, especially with reference to the importance of the haploid or diploid number of chromosomes. He (29) would call this "somatic parthenogenesis." He defines parthenogenesis (p. 11) as

> die apomiktische Entstehung eines Sporophyten aus einem Ei, und zwar (*a*) somatische parthenogenesis, wenn das Ei einen Kern mit der diploiden, unreducierten chromosomenzahl besitzt (*b*) generative parthenogenesis, wenn der Kern des Eies mit der haploiden Chromosomenzahl ausgestattet ist.

He defines apogamy as

> die apomiktische Entstehung eines Sporophyten aus vegetative Zellen des Gametophyten, und zwar (*a*) somatische Apogamie, wenn die Zelle oder der Zellkomplex, die den Sporophyten liefern, in ihren Kerne die diploide Chromosomenzahl besitzen (*b*) generative Apogamie, wenn die Kern der Mutterzellen des Sporophyten nur die Haploide chromosomenzahl fuhren.

That is, WINKLER would call the development, without the aid of a male gamete, of an egg (either haploid or diploid) parthenogenesis, the development of vegetative cells (from a gametophyte) apogamy. There can be no question concerning the latter, but there may be a difference of opinion as to whether the development of both diploid and haploid eggs without fertilization should be called parthenogenesis.

In *Atamosco* the organization of the egg, with the exception of the presence of the diploid number of chromosomes, is perfect so far as morphological evidence goes. The whole egg apparatus is characteristically developed, being quite distinct in appearance from all other cells of the sac. Even the synergids are not to be confused with the egg, each showing its own peculiar characters (figs. 10, 11). It happens that more than one egg is sometimes organized, and apparently all these eggs in *Atamosco*, but no other cell, may function. As all the nuclei of the female gametophyte have the diploid number of chromosomes, it might be thought that any cell could develop a sporophyte, and therefore adventive embryos and polyembryony would be common. But no evidence of adventive embryos was found; and only a few sacs with two embryos were seen, and in every instance they were paralleled in other sacs by an egg in a similar position (figs. 28, 33, 34).

If the egg be considered a diploid gamete, then this should be considered parthenogenesis, but we usually define a gamete as a cell that fuses with another before developing, and it would seem that it should at least be capable of so doing if it is called a gamete. But this egg cell lacks this gamete character; for *Atamosco* seems to show conclusively, not only that it is capable of developing without fertilization, but that it will not fuse with the male gamete although the two nuclei be in contact (figs. 15–24). If this inability to fuse shows that it is really not a gamete, then this is apogamy. It is certainly apogamy if we consider it from the standpoint of the part taken in the process by the male nucleus. Apogamy might be defined as the development of a sporophyte from a diploid egg without fertilization. Vegetative apogamy would be the development of a sporophyte from vegetative cells of the gametophyte, while parthenogenesis would be reserved for the development of a sporophyte from an egg with the haploid number of chromosomes.

In 1900 JUEL (**11**) showed that *Antennaria alpina* has very few staminate individuals, and even these have anthers entirely sterile or only slightly fertile. The megaspore develops without the reduction division. The *Alchemilla* group was investigated by MURBECK (**16**) in 1901 and by STRASBURGER (**23**) in 1905. In the apogamous species the reduction division does not take place in the megaspore development. In the microspore series, in some species, the cells develop no farther than the mother cell stage, others disintegrate after the first division, while still others complete the tetrad division and imperfect pollen is produced. In

1904 OVERTON (18) investigated *Thalictrum*, finding the pollen normal. In some instances there was a reduction division in the development of megaspores, while others developed without this, a case exactly parallel to that of *Marsilia* as shown by STRASBURGER (24) in 1907. In *Taraxacum officinale* JUEL (12, 13) in 1904 and 1905 states that the embryo sac mother cell divides only once, the inner daughter cell developing the sac, but no reduction division takes place. The reduction division does take place in the microspore development.

ROSENBERG (22) in 1907 found in *Hieracium* that the reduction division takes place in the microspore development, but in some species, at least, they are disintegrating even at this time, and the mature anthers are empty. In some species the megaspore development is occasionally normal, giving a haploid gametophyte; but it usually happens that some sporophyte cell (epidermal, chalazal, or nucellar cell) produces the embryo sac. So this is a case of apospory in the development of the sac; and as a diploid egg is organized, it is also a case of apogamy in the development of the embryo. The work of WINKLER (28) in 1904 and of STRASBURGER (26) in 1909 with *Wikstroemia* shows reduction in the microspore development with but little pollen maturing, while reduction does not take place in the megaspore development. In 1910 STRASBURGER (27) added *Elatostema* to the apogamous angiosperms. It also has the reduction in the pollen development with much irregularity in the pollen, some anthers producing pollen of normal appearance. The mother cell of the ovule begins the reduction division but returns to the vegetative mitosis; but in some cases reduction does take place. From these it seems that reduction always takes place in the microspore development, the failure being only in the megaspore series, and in some species it is only partial here.

It will be seen that normal pollen is reported only for *Thalictrum*, some species of *Taraxacum*, and *Atamosco*, the others all having little pollen or defective pollen. The female gametophytes for four genera are diploid, while in the other three both haploid and diploid female gametophytes are developed; but it is not known that these haploid eggs develop parthenogenetically. According to

YAMANOUCHI (30) this is true for the fern *Nephrodium*, which develops a haploid sporophyte from a haploid unfertilized egg.

SUMMARY OF APOGAMOUS ANGIOSPERMS

	Pollen	Female gametophyte
Urticaceae		
Elatostema	Defective	Diploid or haploid
Ranunculaceae		
Thalictrum	Normal	Diploid or haploid
Rosaceae		
Alchemilla Eualchimilla	Defective	Diploid
Thymelaeaceae		
Wikstroemia	Little	Diploid
Compositae		
Antennaria	Little	Diploid
Taraxacum	Normal or little	Diploid
Hieracium	Little	Haploid or diploid
Amaryllidaceae (Leucojaceae)		
Atamosco	Normal	Diploid

STRASBURGER (24) holds that a diploid egg not only is not fertilized, but is not capable of being fertilized. He says (p. 166):

> Womit kann nämlich das apogame Ei einer *Marsilia* besser beweisen, dass es weder Befruchtungsbedurftig noch Befruchtungsfähig ist, als dass es den Spermatazoiden den Eintritt in das Archegonium unmöglich macht? Die diploide Chromosomenzahl bedingt es also, dass im Ei dass Befruchtungsbedürfnis sich nicht einstellt und damit auch der Reiz wegfällt, der die Tätigkeiten sonst anlöst, ist welche die Befruchtung vorbereiten. Also kommt doch wohl eine grundsätzliche Bedeutung an dieser Tatsache zu, dass nicht die einfache, sondern die doppelte Chromosomenzahl im Kern vertreten ist.

In the apogamous *Marsilia* the canal cell does not disintegrate, so that the sperm cannot enter the archegonium. STRASBURGER'S idea of the incapability of the fertilization of the diploid egg was worked out on theoretical grounds altogether, as is shown in the quotations just given, and as he said in discussing this question with me.

OVERTON (18, p. 278) says that in pollinated material he often found pollen tubes in the micropyles of ovules and even in contact with the egg, and also the fusing of the second sperm nucleus with the endosperm nucleus. But his preparations failed to show the fusion of the sperm and egg nuclei, and he adds:

> An diesem Grunde darf ich auch nicht positiv behaupten, dass zur normalen Keimentwicklung stets Befruchtung notwendig ist, wenn auch alle sonstigen Tatsachen und die Beobachtungen an Pflanzen im Freien kienen Zweifel darüber lassen, dass Befruchtung stattfinden kann, wenn das Ei die reduzierte Zahl der Chromosomen führt.

In *Atamosco* I have been able to settle this question beyond a doubt I think; for about 30 cases of an egg dividing with the sperm inactive although in contact, or nearly so, with the egg nucleus were examined. The sperm gradually disintegrates (figs. 18, 21–24, 27) and evidently the egg is incapable of fusing with the sperm, the latter being perfectly normal in every way so far as appearances go, for the sperm had the haploid number of chromosomes, and the pollen seemed entirely normal, and pollen tubes enter the sac in the usual way. The other sperm does fuse with the polars either before or after their fusion. This would seem to show that the triple fusion is not of the same character as that which takes place when the sperm and egg fuse. So *Atamosco* seems to prove STRASBURGER'S theory that the diploid egg is incapable of fertilization.

On the completion of the work, I reported to Professor STRASBURGER what I had found, and in his reply, received February 25, 1912, he says:

> Die Schwerpunk Ihrer Arbeit wird darin liegen, dass der Eikern, weil er doppeltchromosomig ist, die Aufnahme dem Spermakern versagt, ungeachtet die übrigen Einrichtungen bei der Pflanze fortbestehen, die den Spermakern bis in das apogame Ei hineinführen.

Summary

1. The pollen is normal. The haploid number of chromosomes is 12.

2. The third division in the embryo sac shows the diploid number of chromosomes to be 24.

3. Usually the ordinary 8-nucleate sac is organized, but occasionally antipodals organize in the micropylar end and the egg apparatus at the side of the sac, sometimes more than one egg organizing.

4. Two male nuclei come into the sac with the pollen tube, one

fusing with the two polars, the other entering the egg but never fusing with it, and finally disintegrating during the first division in the egg.

5. A diploid egg seems to be incapable of fertilization.

Baylor University
Waco, Texas

LITERATURE CITED

1. Blackman, V. H., On the cytological features of fertilization and related phenomena in *Pinus silvestris*. Phil. Trans. Roy. Soc. London B **190**: 295–427. *pls. 12–14.* 1898.
2. Brown, W. H., and Sharp, L. W., The embryo sac of *Epipactis*. Bot. Gaz. **52**:439–452. *pl. 10.* 1911.
3. Chamberlain, C. J., Oogenesis in *Pinus Laricio*. Bot. Gaz. **27**:268–281. *pls. 4–6.* 1899.
4. Coulter, J. M., Relation of megaspores to embryo sacs in angiosperms. Bot. Gaz. **45**:361–366. 1908.
5. Coulter, J. M., and Chamberlain, C. J., The morphology of angiosperms. 1903.
6. Ferguson, M. C., Life history of *Pinus*. Proc. Wash. Acad. Sci. **6**: 1–202. *pls. 2–24.* 1904.
7. Guignard, L., Sur les anthérozoides et la double copulation sexuelle chez les végétaux angiospermes. Compt. Rend. **128**:864–871. *figs. 19.* 1899.
8. ———, Étude sur les phénomènes morphologiques de la fécondation. pp. 100–147. *pls. 2–5.* 1889.
9. ———, La double fécondation chez les Renonculacées. Jour. Botanique **15**:394–408. *figs. 16.* 1901.
10. ———, La double fécondation chez les Solanées. Jour. Botanique **16**: 145–167. *figs. 45.* 1902.
11. Juel, H. O., Verleichende Untersuchungen über typische und parthenogenetische Fortpflanzung bei der Gattung *Antennaria*. Kgl. Svensk. Vetensk. Akad. **33**: no. 5. pp. 59. 1900.
12. ———, Die Tetradenteilung in der Samenanlage von *Taraxacum*. Arkiv f. Bot. **2**:no. 4. 1904.
13. ———, Die Tetradenteilungen bei *Taraxacum* und anderen Cichoraceen. Kgl. Svensk. Vetensk. Akad. **39**: no. 4. 1905.
14. Land, W. J. G., Double fertilization in Compositae. Bot. Gaz. **30**: 1–11. *pl. 1.* 1900.
15. Modilewski, J., Zur Embryobildung von *Euphorbia procera*. Ber. Deutsch. Bot. Gesells. **27**: 21–26. *pl. 1.* 1909.

16. MÜRBECK, SV., Parthenogenetische Embryobildung in der Gattung *Alchemilla*. Lunds. Univ. Arrsskr. **36**: no. 7. 1901.

17. NAWASCHIN, S., Über die Befruchtungsvorgänge bei einigen Dicotyledoneen. Ber. Deutsch. Bot. Gesells. **18**: 224–230. *pl. 9*. 1900.

18. OVERTON, J. B., Über Parthenogenesis bei *Thalictrum purpurascens*. Ber. Deutsch. Bot. Gesells. **22**: 274–283. 1904.

19. PACE, L., Fertilization in *Cypripedium*. BOT. GAZ. **44**: 353–374. *pls. 24–27*. 1907.

20. ———, The gametophytes of *Calopogon*. BOT. GAZ. **48**: 126–137. *pls. 7–9*. 1909.

20a. ———, *Parnassia* and some allied genera. Bot. Gaz. **54**: 306–329. *pls. 14–17*. 1912.

21. PORSCH, O., Versuch einer phylogenetischen Erklärung des Embryosackes und der doppelten Befruchtung der Angiospermen. Jena. 1907.

22. ROSENBERG, O., Cytological studies on the apogamy in *Hieracium*. Bot. Tidsskr. **28**: 143–170. 1907.

23. STRASBURGER, E., Die Apogamie der Eualchimillen und allgemeine Gesichtspunkte, die sich aus ihr ergeben. Jahrb. Wiss Bot. **41**: 88–164. 1905.

24. ———, Apogamie bei *Marsilia*. Flora **97**: 123–191. *pls. 3–8*. 1907.

25. ———, Chromosomenzahlen, Plasmastrukturen, Vererbungsträger, und Reduktionsteilung. Jahrb. Wiss. Bot. **45**: 1908.

26. ———, Zeitpunkt der Bestimmung des Gaschlects, Apogamie, Parthenogenesis, und Reduktionsteilung. Hist. Beiträge **7**: 1–124. *pls. 1–3*. 1909.

27. ———, Sexuelle und apogame Fortpflanzung bei Urticaceen. Jahrb. Wiss. Bot. **47**: 245–288. *pls. 7–10*. 1910.

28. WINKLER, H., Über Parthenogenesis bei *Wikstroemia indica*. Ber. Deutsch. Bot. Gesells. **22**: 573–380. 1904.

29. ———, Parthenogenesis und Apogamie in Pflanzenreich. pp. 162. *figs. 14*. 1908.

30. YAMANOUCHI, S., Apogamy in *Nephrodium*. BOT. GAZ. **45**: 289–318. *pls. 9–10*. 1908.

EXPLANATION OF PLATES XIII AND XIV

All figures were drawn with a camera lucida. The magnification given is that of the original drawings, which are reduced one-half in reproduction. The abbreviations used are as follows: *e*, egg; *g*, generative nucleus; *m*, male nucleus; *p*, pollen tube; *s*, synergid; *t*, tube nucleus.

FIG. 1.—Pollen grain: tube nucleus (amoeboid) and generative nucleus with 12 chromosomes; ×1000.

Fig. 2.—A spirem in the generative nucleus, from the same anther; ×1000.

FIG. 3.—A germinating pollen grain from the stigma; the generative nucleus in advance of the tube nucleus and in mitosis; ×400.

FIG. 3, *a*.—The generative nucleus from fig. 3; ×1000.

FIG. 3, *b*.—The remainder of the above nucleus from the adjacent section; chromosomes show longitudinal splitting; 12 chromosomes in *a* and *b*; ×1000.

FIG. 4.—Diagram of young ovule showing mother cell; ×600.

FIG. 5.—Two-nucleate sac; daughter nuclei without separating walls; ×400.

FIGS. 6 and 6, *a*.—Two adjacent sections of the same sac; both nuclei of micropylar end of sac in anaphase stage; in each spindle 24 chromosomes can be counted at one end; an accurate count at the other end is not possible; ×1000.

FIG. 7.—Metaphase at chalazal end of four-nucleate sac with 24 chromosomes; ×1000.

FIG. 8.—A somatic cell from ovule walls in metaphase, showing 24 chromosomes; ×1000.

FIG. 9.—Telophase of the third division of the sac; remains of previous spindle; the uncut spindle of the present division shows the beginnings of wall formation; the cut spindle is perpendicular to the section, so only one end of it is shown; this nucleus shows approximately 24 chromosomes; ×1000.

FIG. 10.—The egg apparatus from mature sac; filiform apparatus well developed, but no notch in the synergids; ×1000.

FIG. 11.—Antipodal end of sac; polars in contact; ×400.

FIG. 12.—Micropylar end of sac after pollen tube enters; ×600.

FIG. 13.—Micropylar end of the sac; ×1000.

FIG. 13, *a*.—Primary endosperm of same sac before triple fusion is complete; ×1000.

FIG. 14.—Egg and male nucleus almost in contact; ×1000.

FIG. 14, *a*.—Triple fusion from same sac; ×1000.

FIG. 15.—Micropylar end of sac; ×600.

FIG. 16.—Egg nucleus with well developed spirem and male nucleus near it; ×1000.

FIG. 17.—Egg nucleus showing approximately 24 chromosomes; male nucleus near it; ×1000.

FIG. 18.—Micropylar end of sac; egg nucleus with approximately 24 chromosomes; the nuclear membrane has disappeared; ×1000.

FIG. 19.—Egg nucleus similar to that in fig. 17; ×1000.

FIG. 20.—Egg nucleus similar to that in fig. 18, but sperm still has nuclear membrane; ×1000.

FIG. 21.—Spindle beginning to organize in the egg; male nucleus very dense; ×1000.

FIG. 22.—Metaphase in the egg nucleus; nuclear membrane of the male nucleus breaking down; 1000.

FIG. 23.—Anaphase in egg nucleus; male nucleus has approximately 12 chromatic masses, apparently chromosomes; ×1000.

FIG. 24.—Micropylar end of sac; male nucleus very close to spindle of egg nucleus, which is in anaphase stage; ×600.

FIG. 25.—Undivided egg with about 64-nucleate endosperm; other sacs in this ovary have embryos of about 16 cells; pollen tube contains three nuclei; ×600.

FIGS. 26, 26, *a*, and 26, *b*.—Three sections of the same endosperm nucleus in metaphase; ×1000.

FIG. 27.—Two-celled embryo with pollen tube entering one synergid; ×600.

FIG. 28.—Micropylar end of sac with two eggs and no synergids; ×600.

FIG. 29.—Diagram of sac with three, possibly five, eggs, one antipodal, and two fusing polars; ×105.

FIG. 30.—One antipodal in the usual position, the group of six nuclei in the micropylar end of the sac having the appearance of antipodals; a perfect egg apparatus is organized on one side; one cell near the antipodal region has the egg organization, and four nuclei are fusing in the upper end of the sac; ×105.

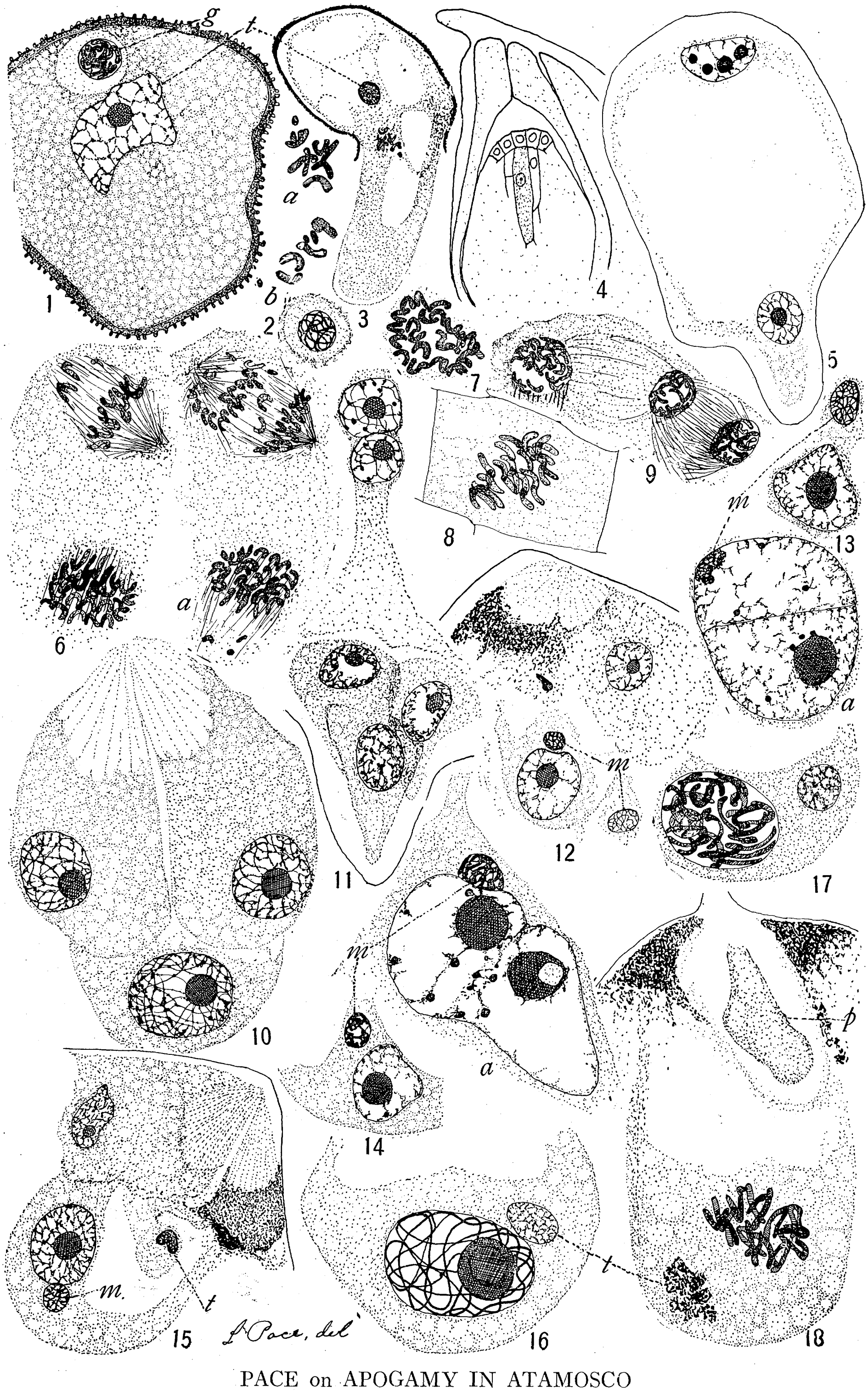
FIG. 31.—Egg apparatus slightly to one side, showing male nucleus in the egg, behind and just in contact with the nucleus; ×600.

FIG. 32.—Egg apparatus of four nuclei near the antipodal region; pollen tube entering; ×1000.

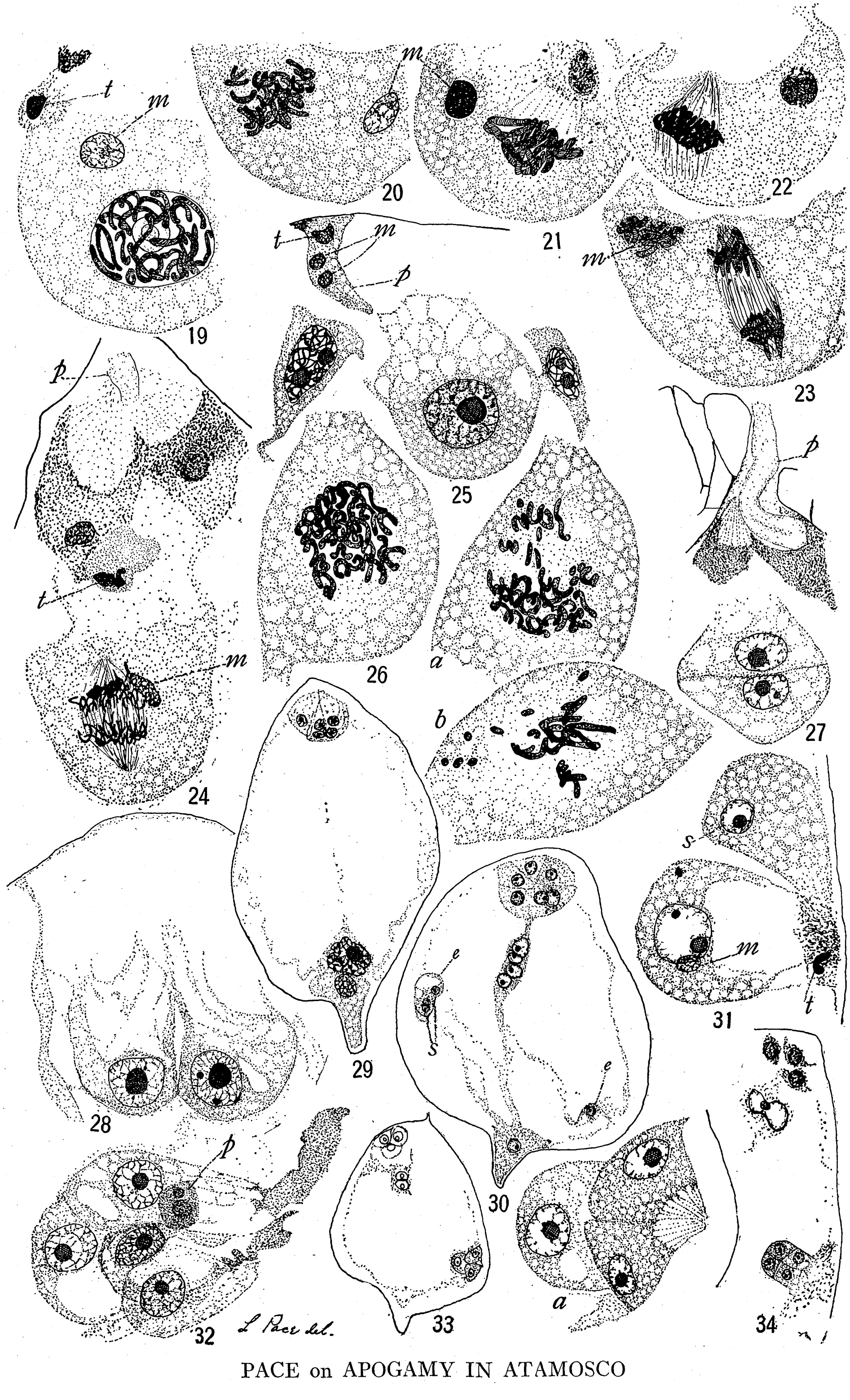
FIG. 33.—The very common type of abnormal sac except that the antipodals are not exactly at the micropylar end of this sac; ×105.

FIG. 33, *a*—Egg apparatus of the same sac; ×600.

FIG. 34.—Four-celled embryo from a side egg; primary endosperm nucleus still undivided; ×105.



PACE on APOGAMY IN ATAMOSCO



PACE on APOGAMY IN ATAMOSCO